

PRIMARY OXIDATION OF SOME ORGANIC COMPOUNDS IN REMOISTENED AIR-DRIED SOIL¹

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Abstract

The effect of adding citrate, caproate, acetate, lactate, alanine, or glutamate on the respiration curves obtained from remoistened soil was studied. The curves can be analyzed as proposed in our experiments with glucose. The curves with caproate and acetate are different from the basic type described with glucose; these two acids inhibited the endogenous respiration. The amount of oxygen consumed during primary oxidation was approximately one-half of the amount necessary for complete oxidation with all substrates studied. Glutamate was most favorable for enzyme synthesis. The oxidation of alanine was very similar to that of lactate. Further study of respiration curves, especially those of acetate and caproate, might be useful in elucidation of soil oxidations.

Introduction

In our previous papers (2, 3, 4) we proposed an analysis of respiration curves of a soil sample treated with glucose. We studied the cumulative curve of oxygen consumption and also the derived curve which showed the course of the respiration rate. The main parameters of these curves are as follows: (1) the amount of oxygen consumed during a primary oxidation, (2) the relative magnitudes of oxidative and assimilative components, (3) the form of the logarithmic increase of the respiration rate during the assimilative part.

We deduced from these parameters some features of the biological component of the soil samples:

(1) From the amount of oxygen absorbed per molecule of the added substrate we deduced what part of the carbon supply was oxidized during the primary oxidation and what part was assimilated to be left in the soil as a carbon source for humus synthesis.

(2) The size of the oxidative component indicated by the initial respiration rate shows the magnitude of the oxidizing enzymatic system originally present in the soil sample (in living cells, or adsorbed on the soil particles), i.e., it is a measure of an actual activity of the microflora.

(3) The course of the logarithmic increase of the respiration rate during the assimilative phase shows the period of latency preceding the elaboration of new enzymes and the rate of this elaboration. These data characterize the potential activity of the microflora.

In all our experiments we used glucose as the substrate. Glucose is readily oxidized by almost all heterotrophic soil microorganisms. Its degree of oxidation, designated ω (2), equals 4, i.e., four electrons are necessary for each carbon atom to bring it to a completely reduced form. The object of the present paper is to ascertain whether the method of analysis previously described can be used for substrates differing from glucose in three ways: (a) they are not so readily oxidized by all microorganisms, (b) their degree of oxidation is

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higher or lower than 4, and (c) their chain is shorter. As examples of such substrates the following were selected for study: citric acid and caproic acid, representing six-carbon compounds with different degrees of oxidation, $\omega = 5$ for the former and $\omega = 2.7$ for the latter; acetic and lactic acids, representing substrates with the same degrees of oxidation as glucose but with shorter molecules; alanine, representing a nitrogen-containing molecule similar to lactic acid; and, finally, glutamic acid representing an amino acid which plays a most important role in nitrogen metabolism of microorganisms.

Only the assimilative part of primary oxidation was studied in the present work, the oxidative part being destroyed by drying.

Materials and Methods

The same meadow sandy-loam reported in our previous papers (3, 4) was air-dried immediately after sampling and stored at 4° C; no changes in respiration were observed during the series of experiments.

Four grams of soil was weighed into a conventional Warburg vessel (area of bottom 11–12 cm²). No difference in respiration was observed using 0.9, 1.0, and 1.1 ml of water; therefore 1.0 ml of substrate solution or water was used. The pH of all substrate solutions was adjusted to 6.0 using 1 *M* KOH, and they were added directly to the soil at zero time. The vessels were incubated at 28° C without shaking. The first reading was made after 1 hour. All results are the mean values of at least four replicate measurements. Statistical treatment was done as follows: From measured values $x_1, x_2, \dots, x_n$ the mean value $\bar{x}$ was determined. The standard deviation s_x and standard error $s_{\bar{x}}$ were calculated in the usual way and, using the *t* test, the necessary difference (at 5% level of significance) was established, $t_4 = 3.182$ or $t_5 = 2.776$, etc. From the values $\bar{x} \pm ts_{\bar{x}}$ calculated for endogenous respiration and from $\bar{x}' \pm ts_{\bar{x}'}$ for total respiration with substrate, the net respiration value for substrate with its necessary difference was calculated as follows:

$$(\bar{x}' - \bar{x}) \pm \sqrt{(ts_{\bar{x}'})^2 + (ts_{\bar{x}})^2}.$$

The fiducial limits are presented graphically in the figures for endogenous respiration and oxidation of acetate. In other cases the level of significance is reported in the text.

Results

The variation of the endogenous respiration rate with time is presented in Fig. 1. It has the same single-peak shape reported by Douglas and Tedrow (1) when they used soil that had been stored air-dry. The peak occurred between the 9th and 15th hours. This endogenous respiration rate has been subtracted from all further data. The endogenous respiration curve was determined four times (always four replicate samples) during the whole period of the experiments. A certain variability, observed in the initial portion of the curve before the maximum was reached, was caused by fluctuation in the time at which the maximum occurred.

Citrate oxidation produced relatively simple curves, which are presented in Fig. 2. On the other hand, the oxidation of caproate, which represents the second six-carbon compound, yielded complex curves which appear in Fig. 3.

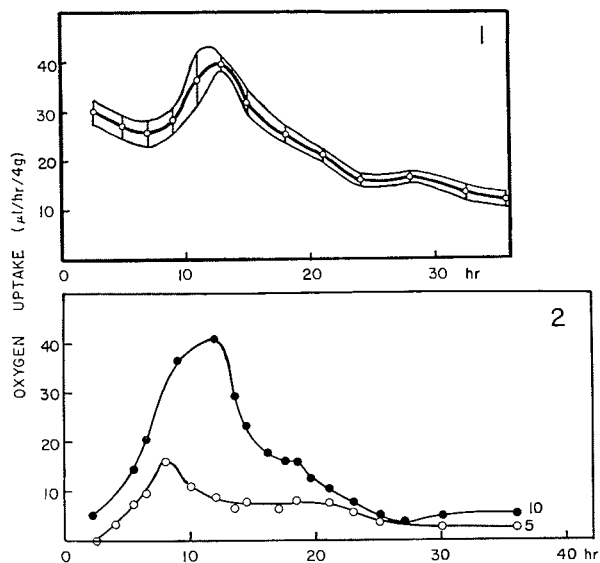


FIG. 1. Endogenous respiration rate of 4 g of remoistened air-dried soil. The limits of significance (5% level) are given as $\pm s.e.t.$

FIG 2. The respiration rates of a soil sample with 5 and $10 \mu\text{M}$ of citrate.

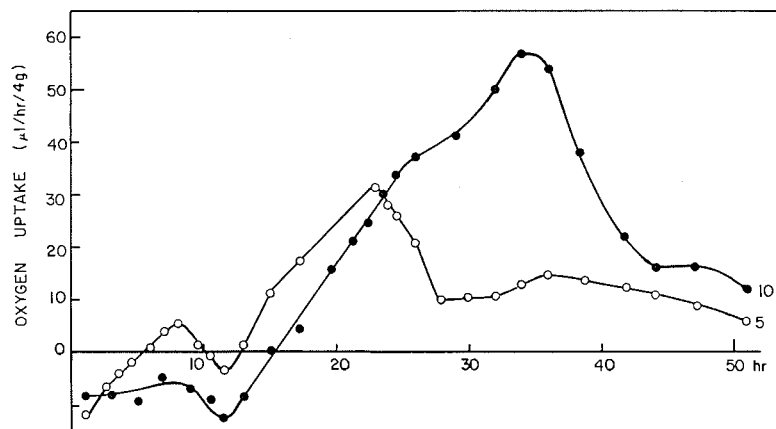


FIG. 3. The respiration rates of a soil sample with 5 and $10 \mu\text{M}$ of caproate.

The distinctive shape of the curves for both concentrations of caproate is significant with the exception of the fluctuations up to the 15th hour observed on the curve of higher concentration. The peak at the 8th hour on the low-concentration curve was significant and was confirmed by several repetitions.

The results obtained with acetate as substrate produced unusually complex curves. The average respiration values with their necessary differences are given graphically in Fig. 4. The first minimum of both curves, shown here at

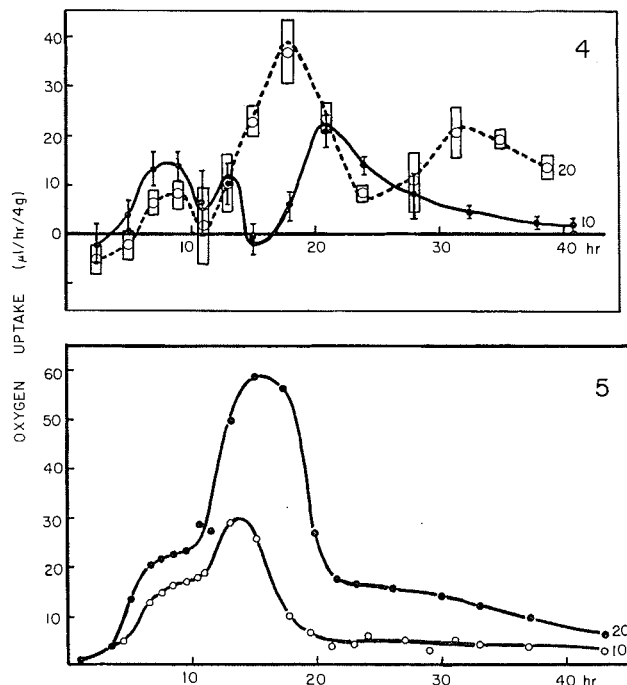


FIG. 4. The respiration rates of a soil sample with 10 and 20 μM of acetate. Significance limits as in Fig. 1.

FIG. 5. The respiration rates of a soil sample with 10 and 20 μM of lactate.

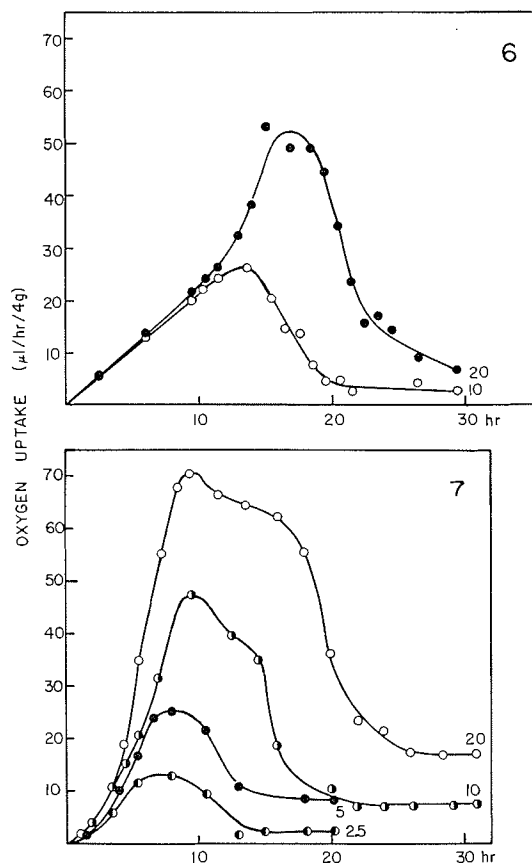
the 11th hour, while not statistically significant, was confirmed by four replicate experiments. However the replications differed slightly in the time and magnitude of the minimum. Also the great variability at 18 and 21.5 hours was caused by fluctuation in the time when the curve started to rise. These examples demonstrate one disadvantage of using the form of statistical analysis on such curves.

The oxidation of lactate is given in Fig. 5. The delay between the 6th and 10th hours is significant. The oxidation curve of alanine (Fig. 6) is very similar to that of lactate. Four concentrations of glutamic acid were used, and corresponding curves are shown in Fig. 7. The oxidation data for all substrates are summarized in Table I.

Discussion

From the results it is apparent that primary oxidation is as well pronounced with these substrates as was shown earlier for glucose (3). The shapes of the respiration curves are often characteristic and their detailed study will likely provide further information about the nature of oxidation processes in the soil.

In the experimental conditions described in the present paper the proportion of the substrates oxidized during primary oxidation is about the same for

FIG. 6. The respiration rates of a soil sample with 10 and 20 μM of alanine.FIG. 7. The respiration rates of a soil sample with 2.5, 5, 10, and 20 μM of glutamate.

each (see final column, Table I). The error using smaller concentrations is relatively high. The results with greater concentrations of substrates do not show any significant difference, with the exception of the greatest concentration of glutamate. All substrates are approximately 47% oxidized. This value is much higher than those obtained for glucose in the previous papers, namely 24.5% and 27.6%. We suppose that glucose is the best substrate for assimilation and therefore its assimilation is much greater than its oxidation.

Although the values summarized are roughly uniform, the shape of the respiration curves is very characteristic. Those for citrate, alanine, and glutamate have the simple basic form as previously described for glucose, differing only in the logarithmic rise of respiration rate; glutamate stimulated the respiration most of all. The logarithmic rise is characterized by an equation:

$$Q_{O_2} = 2^{(1/1.07)t}$$

Corresponding expressions for respiration rate for citrate and alanine are:

TABLE I
Relative oxidation of substrates during primary oxidation calculated from oxygen consumption*

Substrate	Concn., μM	Max. resp. $c, \dagger$ hours	Prim. oxid. $d, \ddagger$ hours	Oxygen uptake (at time d)			
				Endogenous, μl	Substrate, net, μl	Mole ratio $O_2/\text{substr.}$ M/M	Relative oxidation, %
Citrate	5	9	16	498.0 ± 23.3	187.4 ± 41.4	1.68 ± 0.093	37.4 ± 8.2
	10	12	25	688.1 ± 31.8	474.7 ± 51.1	2.12 ± 0.218	47.4 ± 5.1
Caproate	5	23	28	738.4 ± 34.7	268.8 ± 59.2	2.40 ± 0.133	30.0 ± 6.6
	10	34	44	934.2 ± 36.1	810.0 ± 39.9	3.61 ± 0.178	45.1 ± 2.2
Lactate	10	14	20.5	609.5 ± 27.9	275.8 ± 44.2	1.23 ± 0.197	41.0 ± 6.6
	20	16	22	641.5 ± 29.2	621.1 ± 46.0	1.39 ± 0.205	46.2 ± 3.4
Alanine	10	13	21	620.2 ± 28.3	295.9 ± 48.3	1.32 ± 0.215	44.0 ± 7.2
	20	17	24	672.6 ± 31.0	620.0 ± 56.6	1.38 ± 0.253	46.1 ± 4.2
Glutamate	2.5	7	13	394.8 ± 20.3	104.2 ± 31.1	1.86 ± 0.550	41.7 ± 12.4
	5	8	14	434.4 ± 21.3	225.8 ± 40.6	2.01 ± 0.360	45.2 ± 8.1
	10	9.5	22	641.5 ± 29.2	487.6 ± 34.8	2.17 ± 0.156	48.8 ± 3.5
	20	9.5	26	705.1 ± 32.6	1037.0 ± 58.5	2.31 ± 0.131	51.8 ± 2.9

*Data are given as mean value $\pm$ necessary difference at 5% point.

$\dagger$ The time of maximum respiration rate.

$\ddagger$ The time of the end of primary oxidation.

$Q_{O_2} = 2.14 \times 2^{(1/2.02)t}$ and $Q_{O_2} = 5.63 \times 2^{(1/4.94)t}$, respectively, i.e., the "mean doubling time" is approximately twofold longer for the former and fivefold longer for the latter.

The delay in the respiration for lactate and the form of the respiration curves for acetate and caproate cannot as yet be explained. Only the second peak in the respiration for acetate is dependent on the substrate concentration. This unusual shape occurred only with soil that had undergone air-drying. The respiration curves obtained when the soil had not been air-dried gave a simple form whether acetate, caproate, or the other substrates were used.

An interesting finding was the depression of oxygen consumption for the first 4 hours when acetate or caproate was added to remoistened soil, as shown in Table II.

TABLE II
Effect of acetate and caproate on the reactive oxidation of air-dried and remoistened soil

Substrate added	Respiration by soil sample in $\mu\text{l O}_2$ per hour per 4 g*		
	Maintained moist	Air-dried and remoistened	Reactive oxidation†
None	21.92 ± 1.72	30.09 ± 2.45	8.17
Acetate, 20 μM	30.00 ± 2.67	25.19 ± 1.43	3.27
Caproate, 5 μM	38.00 ± 0.47	26.65 ± 1.68	4.73

*The respiration rate is calculated from the first 4 hours. The mean values and necessary difference at 5% point are given.

†I.e., the difference between the endogenous respiration of air-dried and remoistened soil and the endogenous respiration of soil which had not been air-dried. The substrates which had been added to air-dried and remoistened soil were not oxidized during the first 4 hours; therefore the respiration in this interval can be compared with the endogenous respiration of the moist sample.

The air-drying and remoistening stimulated the endogenous respiration by about 36%. We call this addition "reactive oxidation". When acetate and caproate are added to soil treated in this way this reactive oxidation was suppressed to about 40% and 58%, respectively, of that occurring in soil without any substrate. This peculiar effect of these compounds might be used to study the nature of reactive oxidation.

References

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